d.) Remarks

Applicant has canceled claims 59 and 64-67, amended claims 58 and 63, and added new claim 68. Support for the amendments to claim 58 can be found in the specification at page 14, lines 28-29. Support for the amendments to claim 63 can be found in the specification at page 14, line 26. Support for new claim 68 can be found in the specification at page 15, lines 1-2. Accordingly, no new matter or new issues are introduced with these amendments and the new claim, and claims 1-26, 40-58, 60-63 and 68 are currently pending.

Remarks Regarding 35 U.S.C. § 102(b)

Claims 58, 60 and 62 stand rejected, under 35 U.S.C. § 102(b), as allegedly anticipated by Teni et al. ("Teni"). Applicant respectfully traverses this rejection.

In the Office Action (Paper No. 14), it is alleged that Teni discloses measuring the level of a prostatic inhibin-like peptide ("PIP") and that, in view of applicant's broad definition of the inhibin protein, it must be concluded that PIP is encompassed within the meaning of inhibin in the claims. In applicant's Amendment filed and dated September 26, 2002 (Paper No. 13), applicant asserted that PIP was not an inhibin protein and cited to an abstract of an article by Gorden et al. ("Gordon"), which indicates that Beta-microseminoprotein ("β-MSP") is not an inhibin (*see* Title). In Paper No. 14, the examiner requests a copy of the Gordon abstract and further states that the relation between PIP and β-MSP is unclear.

As requested, enclosed please find the Gordon abstract and also the full length Gordon publication (Biology of Reproduction 36:829-35, 1987). As indicated in Gordon, the protein identified as β-MSP is not believed to be an inhibin because β-MSP and tryptic peptides of β-MSP failed to depress follicle-stimulating hormone ("FSH") levels in the medium at any of the doses tested (10-10,000 ng/ml). However, under the same conditions, partially purified inhibin

from porcine follicular fluid ("pFFl") showed a dose-dependent inhibition of FSH secretion with a 50% inhibitory dose at 50 ng, which paralleled that seen with standard inhibin (*see* Gordon abstract, discussion, and Figure 4).

Applicant further notes that the relationship between PIP and β-MSP is disclosed in Gordon and Teni. In Teni, PIP is stated to contain a known amino acid sequence as disclosed in reference number 11, which is Seith et al. (FEBS Letters 175:349-55, 1984). In Gordon, reference is made to a separate preparation by Seth et al. (*see* page 830, second column), which is the same publication referred to by Teni (FEBS Letters 175:349-55, 1984). Gordon notes that the sequence identified as β-inhibin by Seth and others was determined to be structurally identical to a sperm-coating antigen and designated as β-MSP. In other words, β-MSP is also known as PIP, which applicant further represents has no sequence homology to applicant's claimed inhibin protein. Further, this protein is not a part of the TGF-β superfamily that encompasses applicant's claimed inhibin protein.

Applicant respectfully asserts that PIP and β-MSP are in fact the same protein and that β-MSP is not an inhibin protein of the claimed invention. Accordingly Teni does not disclose or suggest the claimed invention.

Thus, the rejection of claims 58, 60 and 62, under 35 U.S.C. § 102(b), is overcome and applicant respectfully requests that it be withdrawn.

Remarks Regarding 35 U.S.C. § 103(a)

Claims 58, 60, 62 and 63 stand rejected, under 35 U.S.C. § 103(a), as allegedly obvious over Ying et al. ("Ying") and Teni. Applicant respectfully traverses this rejection.

As discussed above, Teni does not disclose or suggest applicant's inhibin protein. Teni relates to a different protein, β-MSP, which is in fact not an inhibin protein of the claimed

invention. Thus, absent Teni this rejection cannot stand. Nevertheless, in an effort to expedite prosecution, applicant offers the following remarks regarding the relevance of Ying.

The claimed invention is based on the surprising observation that the level of inhibin protein in a mammal can be used to determine the presence or absence of prostate cancer. However, as is stated in Ying, the authors do not consider human prostate cancer cells to express inhibin (*see* Ying, page 400, bottom of page: "This observation [the identification of inhibin in normal rat prostatic epithelial cells] is different from that in human cancer cells (13-15) which have no inhibin reactive cells."). Therefore, Ying could not come to the conclusion that a level of inhibin protein can correlate with prostate cancer. Accordingly, Ying actually teaches away from the claimed invention, which is strong evidence of unobviousness.

Thus, the rejection of claims 58, 60, 62 and 63, under 35 U.S.C. § 103(a), is overcome and applicant respectfully requests that it be withdrawn.

Remarks Regarding 35 U.S.C. § 112, Second Paragraph

Claims 58, 60, 62 and 63 stand rejected, under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Applicant respectfully traverses this rejection.

With regard to claims 58 and 63, it is allegedly unclear what is encompassed by the term "modified." As is believed clear to one of ordinary skill in the art, modified has a plain meaning which in context here means a change in the level of inhibin protein. As is recited in claim 58, this change is between a mammal with prostate cancer and a normal mammal without prostate cancer. As this term is believed to be clear, this aspect of the rejection is believed overcome.

With regard to claim 63, it is allegedly unclear what is intended by the term "absent." Solely in an effort to expedite prosecution, the term absent has been deleted from claim 63. However, new claim 68 has been added to make it clear that a reduction of inhibin protein level

in the mammal encompasses "an absence" of detectable inhibin protein. Thus, this aspect of the rejection is moot.

Thus, the rejection of claims 58, 60, 62 and 63, under 35 U.S.C. § 112, second paragraph, is overcome and applicant respectfully requests that it be withdrawn.

Conclusion

The application is believed to be in condition for allowance and the prompt issuance of a Notice of Allowance is respectfully requested. In addition, if the application is considered to be in condition for allowance, applicant respectfully request that the species restriction imposed in the Office Action, mail dated March 9, 2001 (Paper No. 9), be withdrawn and that claim 61, the one claim that was subject to the species restriction, be included in the application and amended in accordance with similar amendments made to claim 62 herein.

If there are any fees due with the filing of this Response, including any fees for an extension of time, applicant respectfully requests that extension and requests that any and all such fees be charged to the undersigned's Deposit Account No. 03-1952.

Respectfully submitted,
Morrison & Foerster LLP

Dated: January 7, 2004

By: _____
James Romenick
Registration No. 36,902
For: Barry E. Bretschneider
Registration No. 28,055

Enclosed: Gordon abstract
Gordon publication

Customer I.D. No. 25227
Morrison & Foerster LLP
Suite 300, 1650 Tyson's Boulevard
McLean, VA 22102
Tel: (703) 760-7700
Fax: (703) 760-7777

BIOLOGY of REPRODUCTION

Biology of Reproduction, Vol 36, 829-835, Copyright © 1987 by Society for the Study of Reproduction

ARTICLES

Beta-microseminoprotein (beta-MSP) is not an inhibin

WL Gordon, WK Liu, K Akiyama, R Tsuda, M Hara, K Schmid and DN Ward

Beta-microseminoprotein (beta-MSP), a sperm-coating antigen isolated from human seminal plasma, has apparent structural identity with "beta- inhibin" isolated from the same source. Publication of the amino acid sequence of beta-MSP revealed a greater than 96% homology with "beta- inhibin," with only a proline-threonine substitution at positions 39 and 40, and the omission of a glycine at position 93. Due to the nearly identical sequences of "beta-inhibin" and beta-MSP, we examined the ability of beta-MSP and its tryptic peptides to inhibit basal follicle- stimulating hormone (FSH) secretion from rat pituitary cells in culture, the inhibin bioassay. Whole pituitaries collected from 250- to 300-g male rats were dispersed enzymatically and plated onto 24-well culture dishes for 3 days. beta-MSP and its tryptic peptides were dissolved in cell culture medium, applied to the pituitary monolayer cell cultures, and incubated for an additional 3 days. FSH levels in the medium were determined by radioimmunoassay. A partially purified preparation of inhibin and our in-house inhibin standard, both prepared from porcine follicular fluid (pFFl), were included in the same assay. Whereas the partially purified inhibin from pFFl showed a dose- dependent inhibition of FSH secretion, with a 50% inhibitory dose (ID50) of 50 ng, which paralleled that of the standard, beta-MSP and its tryptic peptides failed to depress FSH levels in the medium at any of the doses tested (10-10,000 ng/ml). We conclude that beta-MSP is not an inhibin under our assay conditions.

This article has been cited by other articles:

- Lazure, C., Villemure, M., Gauthier, D., Naude, R. J., Mbikay, M. (2001). Characterization of ostrich (Struthio camelus) {beta}-microseminoprotein (MSP): Ideication of homologous sequences in EST databases and analysis of their evolution during speciation. *Protein Sci* 10: 2207-2218 [Abstract] [Full Text]
- Mäkinen, M., Valtonen-André, C., Lundwall, A. (1999). New World, but not Old World, monkeys carry several genes encoding {beta}-microseminoprotein. *Eur J Biochem* 264: 407-414 [Abstract] [Full Text]

These three studies also included amino acid sequence data limited to the N-terminal sequence of the two chains of the molecule, but consistent with the complete structure of inhibin predicted by Mason et al. (1985). The relationship of these preparations to that of Rivier et al. (1984), also from pFFl, but with a molecular weight of 12,000 and a reported potency approximately 5-fold greater than the 32,000 molecular weight preparations cited above is not clear. However, in a subsequent report, Rivier et al. (1985) explain, "when also considering the large number of Cys residues one may suggest a globular structure which would explain the low apparent MW on gel permeation and its MW of 25K when nonreduced as compared to 32K, the sum of the low MW of the two denatured chains on SDS-PAGE." The 25,000 estimate appears to be a revision of their earlier estimate.

There is also a recent report of an inhibin preparation from bovine follicular fluid (Robertson et al., 1985) that has a specific activity comparable to that found for porcine inhibin (Ling et al., 1985; Miyamoto et al., 1985; Rivier et al., 1985; Gordon et al., 1986). The molecular size, however, is slightly larger (56,000) and the N-terminal sequences (six residues) are different (Robertson et al., 1985). However, this same group (Robertson et al., 1986) have recently shown a 31,000 molecular weight inhibin derived from the 56,000 molecular weight inhibin can also be obtained from bovine follicular fluid. That the 32,000 form of inhibin is a proteolytic fragment of the 56,000 form of inhibin is further implied by the elucidation of the cDNA structure of inhibin from the bovine (Forage et al., 1986).

In contrast to the substantive reports on female-derived inhibin (Mason et al., 1985; Forage et al., 1986), the claims for sequences of proteins or peptides from the male with "inhibin-like activity" have failed to receive confirmation from laboratories other than those advancing the claims. When we first reviewed these claims (Channing et al., 1985), we were very much encouraged that inhibin research was entering a phase during which specific structures were being proposed. However, developments since that time lead us to conclude that the relationship of these specific peptides derived from human seminal plasma to inhibin or inhibin activity remains to be established. Ramasharma et al. (1984) reported a 31-amino acid peptide obtained from seminal plasma that had inhibin-like activity. The structure was confirmed by synthesis (Yamashiro et al., 1984). The authors noted that their preparation was active only under certain assay conditions (short-term incubation) in selected systems (Li et al., 1985), but was inactive in long-term incubations such as those often used to measure inhibin activity (e.g., Channing et al., 1985). The sequence of the predominant basic protein in liquified human seminal plasma was subsequently reported by Lilja and Jeppsson (1985). This 52-residue protein has the same N-terminal sequence as the 31-amino acid residues of the inhibin-like peptide of Ramasharma et al. (1984).

The separate preparation of Seidah et al. (1984) designated "β-inhibin," also from seminal fluid, was sequenced to completion (94 residues). It was subsequently claimed that the C-terminal portion of this protein (28 residues) was the "active core" of "β inhibin" and accounted for all the activity (Arbatti et al., 1985). This report and that of Seidah et al. (1984) present assay data to accompany the sequence studies on "β-inhibin." The C-terminal peptide was designated "β_2-inhibin." A. R. Sheth of Bombay is a collaborator on the sequence studies of Seidah et al. (1984) and Arbatti et al. (1985) of Montreal, and on the studies from Jornvall's laboratory in Stockholm (Johansson, 1984). The latter group sequenced "β-inhibin" (Johansson et al., 1984) and pointed out that the sequence was apparently identical to the structure of a sperm-coating antigen. The amino acid sequence of this antigen, designated β-microseminoprotein (β-MSP), was described in detail by some of the present authors (Akiyama et al., 1985). Thus, it was of interest to test the inhibin potency of an authentic β-MSP preparation directly. This report will demonstrate that β-MSP has no detectable inhibin activity in a pituitary cell culture assay system used to follow the fractionation of inhibin from pFFl (Gordon et al., 1986).

MATERIALS AND METHODS

Preparation of β-MSP and Tryptic Peptides

β-MSP was isolated and purified by the method of Akiyama et al. (1985). Tryptic peptides were prepared by incubating β-MSP with TPCK (L-1-tosylamide-2-phenylethylchloromethyl ketone)-trypsin in 100 mM ammonium bicarbonate, pH 8.0, for 16 h at 37°C. After incubation, the digest was fractionated over a column of Sephadex G-25 fine (Pharmacia Fine Chemicals, Piscataway, NJ) in 50 mM ammonium bicarbonate. Under these conditions, the

N-terminal (1—66 amino acids) fragment (β-MSP-T-1) was separated from the putative C-terminal portion (β-MSP-T-2).

Amino acid analyses were performed on an LKB Model 4400 automated analyzer using ninhydrin for detection. Samples were hydrolyzed for 24 h at 110°C in sealed tubes under vacuum with 6 N constant boiling HCl containing 0.1% phenol. N-terminal amino acid analysis was done according to the method of Hartley (1976).

Preparation of Partially Purified Inhibin from Porcine Follicular Fluid

A partially purified inhibin preparation was isolated from pFFl essentially as described previously (Gordon et al., 1986). In this procedure, raw follicular fluid was acetone-precipitated at −20°C (Huang and Miller, 1984), added to 95% glacial acetic acid at room temperature, and stirred overnight at 4°C. The residue was removed by centrifugation. The supernatant was precipitated overnight with 90% ethanol at 4°C. The ethanol precipitate was dissolved in water, dialyzed against water, and lyophilized. Twelve grams of this preparation, designated EP, were dissolved in 126 mM ammonium bicarbonate and fractionated at 500 ml/h through Sephacryl S-200 (Pharmacia) in a 14 × 95-cm column (Amicon-Wright, Danvers, MA). The eluted fractions were concentrated on a Minitan Filtration Unit (Millipore Corp., Bedford, MA) using 10,000 molecular weight cut-off membranes. The S-200D fractions from two such S-200 chromatographies were pooled (250 mg) and applied to a Sephadex G-75 column (2.5 × 135 cm) equilibrated in 25% acetic acid. The eluted fractions were dried two times by lyophilization to remove acetic acid, and assayed for inhibin activity.

Inhibin Bioassay

The inhibin bioassay was conducted as previously described employing young adult male rats (250—300 g) to obtain pituitaries for a basal FSH release assay from pituitary cell-monolayer culture (Channing et al., 1985). The FSH concentrations in the medium were analyzed by one-way analysis of variance using the Minitab program (Ryan et al., 1985) as adapted to the Cyber 174 computer. The probability level for a least significant difference was taken as 0.05.

RESULTS

Preparation of β-MSP and Its Tryptic Peptides

Figure 1 shows the elution profile for the tryptic peptides of β-MSP. Tryptic peptides T-1 and T-2 yielded sufficient quantities of material for further analysis, but T-3, T-4, and T-5 did not. The N-terminal amino acids in T-1 were serine and lysine (Table 1), and the amino acid composition (data not shown) indicated that these peptides contain predominantly the N-terminal 66 amino acids. T-2 was a mixture of peptides with N-terminal (Table 1) of lysine, isoleucine, valine, glycine, aspartic and glutamic acids, and threonine. Traces of phenylalanine and leucine were also detected. These amino acids, with the exception of valine, phenylalanine, and leucine, are on the C-terminal side of a lysine or arginine in the β-MSP sequence and would be predicted to be N-terminal after tryptic digestion. Amino acids that are C-terminal to an arginine or lysine in the β-MSP sequence,

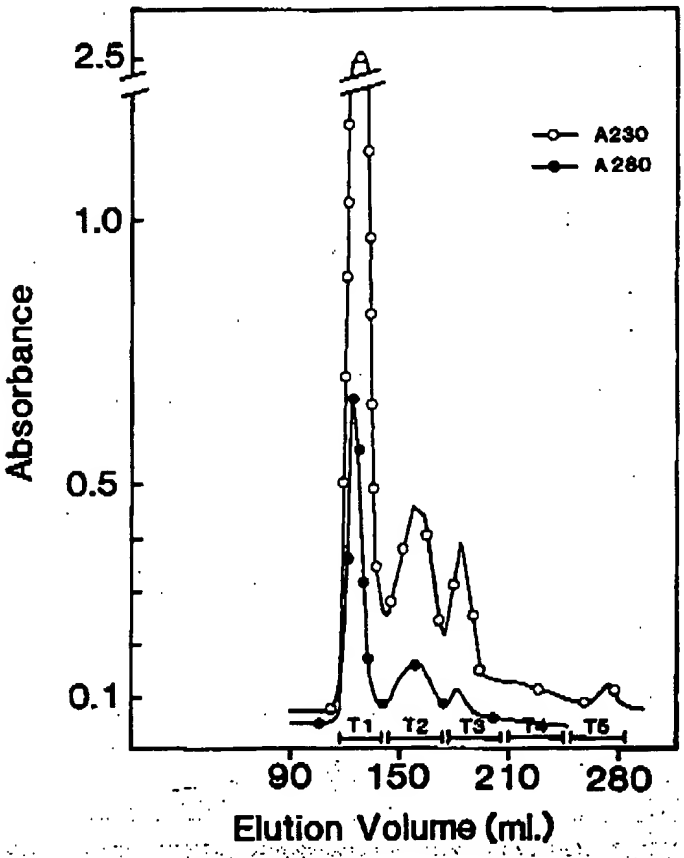

FIG. 1. Elution profile of the 16-h tryptic digest of β-MSP (beta-microseminoprotein) on Sephadex G-25 Fine in 0.05 M ammonium bicarbonate, pH 7.8, at a flow rate of 10 ml/h. *T-1, T-2, T-3, T-4,* and *T-5* indicate peptide fractions pooled for further analysis.

TABLE 1. N-terminal amino acid analysis of β-MSP tryptic peptide fractions T-1 and T-2.

Peptide	DNS-amino acid*
T-1	Serine, lysine
T-2	Lysine, isoleucine, valine, glycine, aspartic acid, glutamic acid, threonine (phenylalanine, leucine)**

*DNS-amino acids = dansylated amino acid derivatives indicative of N-termini of peptides.

**Trace amounts of phenylalanine and leucine were detectable.

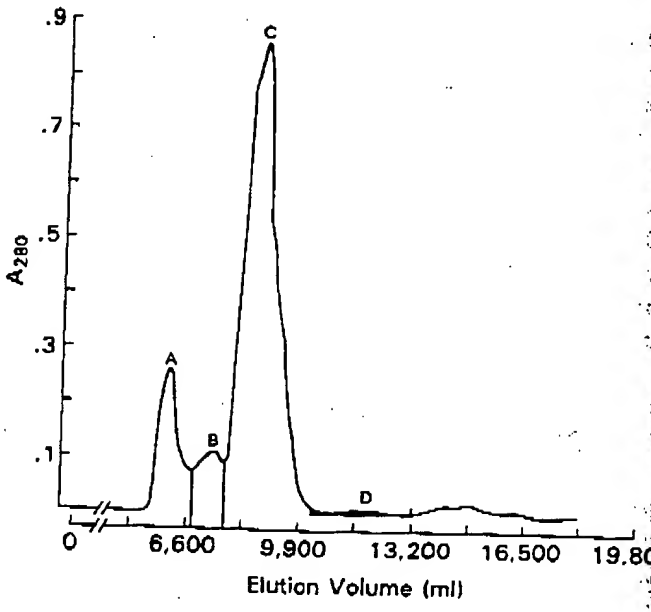

FIG. 2. Elution profile of EP (material obtained from acetone/acetic acid extraction of porcine follicular fluid) on a 14 × 95-cm Sephacryl S-200 column equilibrated at 4°C in 0.126 M ammonium bicarbonate at a flow-rate of 500 ml/h. The *letters* indicate the fractions that were collected and lyophilized. The *darkened area* indicates the inhibin fraction used for further fractionation on Sephadex G-75.

but were not detected in N-terminal analysis of T-1 or T-2, were tyrosine-75, histidine-26, and half-cystine-18. These amino acids were probably protected from tryptic digestion in the nonreduced β-MSP substrate. It was clear from the N-termini that fraction T-2 contained the majority of the predicted tryptic peptides beyond the large N-terminal peptide (T-1). To conserve materials and assay effort, we looked for inhibin activity in this fraction without further resolution.

Partial Purification of Inhibin from pFFl

Partial purification of the pFFl inhibin was as summarized in Table 2. This fractionation was comparable with our earlier report (Gordon et al., 1986), except the sample size and columns were proportionately scaled-up (Fig. 2), and the Sephadex G-75 step was added (Fig. 3), as noted in the Materials and Methods section. Recovery of inhibin activity was virtually quantitative at each step. The 5.8% recovery for the S-200D fraction (Table 2) represents the activity in the 32,000 molecular weight range. The balance of the activity was in fractions B and C and represents higher molecular weight material. Thus, the Sephacryl S-200 step did not introduce significant loss of activity.

Inhibin Biological Activity of β-MSP and Its Tryptic Peptides

The inhibin biological activities of β-MSP, its tryptic peptides, and the partially purified porcine ovarian inhibin are summarized in Figure 4. Whereas the partially purified inhibin preparation from pFFl showed a dose-response curve parallel to our porcine ovarian

TABLE 2. Summary of inhibin biological activity in the fractionation of porcine follicular fluid (pFFl).

Preparation*	Dry weight (mg)	ID_{50} (ng)	Sp. act. (units/mg)	Total activity (units)	Activity recovery (%)**
EP	30,000	2500	2.4	72,000	100
S-200D‡	250	500	12	4,200	5.8
G-75B	34.8	50	120	4,200	100

*EP refers to the material obtained after acetone/acetic acid extraction of pFFl; it was used as the starting material for the Sephacryl S-200 column. S-200D refers to the D fraction obtained from Sephacryl S-200 chromatography of EP. G-75B refers to the B fraction obtained from Sephadex G-75 chromatography of S-200D.

**The recoveries are compared to the total activity applied to the column.

‡The combined recovery of inhibin activity in Fractions A, B, and C range from 52.5–89.2% of the total activity applied in a series of chromatographic runs.

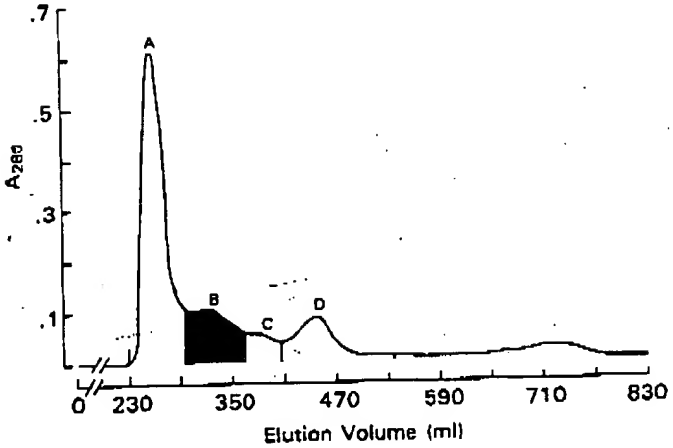

FIG. 3. Elution profile of the D fraction from the S-200 column on Sephadex G-75 equilibrated in 25% acetic acid at 4°C with a flow-rate of 70 ml/h. The *letters* indicate the fractions pooled and lyophilized. The *darkened area* indicates the inhibin-active fraction.

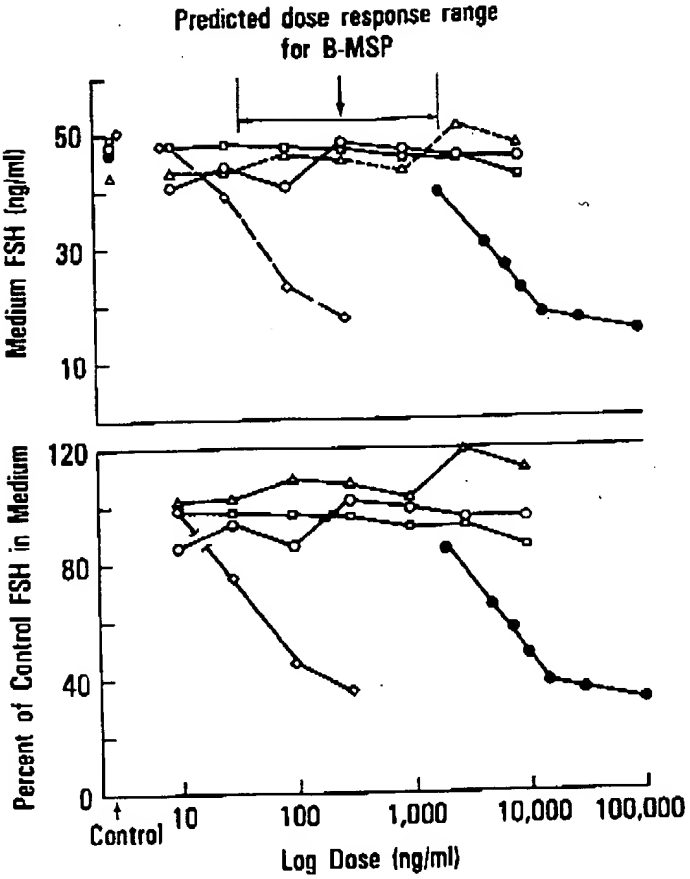

FIG. 4. Inhibin bioassay of β-MSP (beta-microseminoprotein) and pFFI (porcine follicular fluid) inhibin preparations in the assay of basal FSH released by rat pituitary cells in culture. Each *value* in the *upper panel* represents the mean FSH concentration from triplicate culture wells assayed in duplicate by RIA. The *lower panel points* represent a transformation of the means in the upper panel to percentage of control FSH levels in the cell culture medium. The *horizontal arrow* indicates the predicted dose-response range for β-MSP based on the dose-response range of β-inhibin in the pituitary cell culture assay (Arbatti et al., 1985). The symbols are: □, β-MSP; ○, T-1; △, T-2; ●, HSN std inhibin; ◇, G-75B partially purified pFFI inhibin.

standard, the β-MSP preparations showed no convincing depression in medium FSH levels at any dose level tested. One-way analysis of variance indicated that there was no significant difference in the FSH concentrations in the medium at any dose level of β-MSP (10—10,000 ng/ml) or its tryptic peptides. Figure 4 also indicates the dose-response range for "β-inhibin" as reported by Arbatti et al. (1985). In assigning this range, we converted their "μmole" designation to ng based on the molecular mass for β-MSP of 10,704 daltons. Within this range, we found no significant depression in mean FSH concentration from control levels in the media from mean FSH concentrations in the wells for β-MSP or the tryptic peptides.

DISCUSSION

We examined the inhibin biological activity of β-MSP in our in vitro pituitary cell culture bioassay. There was no statistically significant evidence to indicate β-MSP contained any inhibin biological activity. We conclude, therefore, that β-MSP is not inhibin. We also examined the tryptic peptides of β-MSP and did not find any biological activity among these peptides. A similar treatment had been claimed by Arbatti et al. (1985) to yield "β_2-inhibin" from "β-inhibin," although they tested only a synthetic peptide equivalent. As controls on our own assay conditions, highly significant FSH suppression with good dose-response relationship was obtained with a laboratory reference preparation from porcine follicular fluid, as well as a partially purified inhibin from this same source.

Failure to observe biological activity among the β-MSP preparations could be due to a number of reasons. First, the degree of homology between "β-inhibin" and β-MSP is not 100%; the points of difference were noted in the introduction. If these amino acid sequences are indeed correct, then the absence of the half-cystine at position 40 in β-MSP is not a trivial one since 1/2-Cys residues are generally conserved among homologous protein families; see, for example, the gonadotropin family (Gordon and

Ward, 1985) and the relaxin families (Kemp and Niall, 1984).

The failure to observe biological activity in the tryptic peptides could be due to the cleavage of essential lysine or arginine C-terminal bonds that may be critical to biological activity. This could well be the case, since our N-terminal studies on these tryptic peptides indicate β-MSP showed considerably greater tryptic proteolysis (Table 1) than that implied for "β_2-inhibin" in the studies of Arbatti et al. (1985). However, this point is difficult to evaluate since their data are not presented and the tests for biological activity that they did run were on a synthetic replica of this part of their "β-inhibin" (Arbatti et al., 1985).

Finally, one further possibility must be considered. When two proteins (e.g., "β-inhibin" and β-MSP) are isolated from the same source and differ only by the slightest differences suggested by the sequence data available for these two proteins, there is the consideration that sequencing errors may be involved rather than true protein differences. In this connection, the two groups sequencing the putative "β-inhibin" checked each other quite well (Johansson et al., 1984; Seidah et al., 1984) although Johansson et al. did indicate some technical difficulty with the last three residues at the C-terminus, an area accounting for one of the three differences in the sequence proposed for β-MSP (Akiyama et al., 1985). For this reason, we are currently re-examining the β-MSP sequence.

Whatever might be the ultimate conclusion for "β-inhibin" or "β_2-inhibin" biological activity (at this time, we are unaware of any confirmation of potency by other investigators), it is very clear from the present study that an authentic sample of β-MSP, in spite of a very high sequence homology (at least 96.8%) to "β-inhibin," is not inhibin under conditions where a bona fide inhibin preparation showed a good dose-response regression.

ADDENDUM

While this manuscript was under review, Kohan et al. (1986) reported that an authentic sample of "β-inhibin" or the synthetic C-terminal 28-residue, "β_2-inhibin," were devoid of inhibin activity. That report, together with the present report, makes it clear that any inhibin activity in seminal plasma or rete testis fluid cannot be ascribed to "β-inhibin," "β_2-inhibin," or β-MSP protein.

ACKNOWLEDGMENTS

We would like to thank Dr. Gabriel Bialy of the National Institute of Child Health and Human Development for providing the porcine follicular fluid. Additionally, we would like to thank the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases for providing the rat FSH RIA kit. We would also like to thank Ms. Nancy Jones for excellent technical assistance, and Mrs. Harriette Young for typing the manuscript.

REFERENCES

Akiyama K, Yoshioka Y, Schmid K, Offner GD, Troxier RF, Tsuda T, Hara M, 1985. The amino acid sequence of human β-microseminoprotein. Biochim Biophys Acta 829:288–94

Arbatti NJ, Seidah NG, Rochemont J, Escher E, Sheth AR, Chretien M, 1985. β_2-Inhibin contains the active core of human seminal plasma β-inhibin: synthesis and bioactivity. FEBS Lett 181:57–63

Channing CP, Anderson LD, Hoover DJ, Kolena J, Olsteen KG, Pomerantz SH, Tanabe K, 1982. The role of non-steroidal regulators in control of oocyte and follicular maturation. Recent Prog Horm Res 38:331–408

Channing CP, Gordon WL, Lu W-K, Ward DN, 1985. Minireview: physiology and biochemistry of ovarian inhibin. Proc Soc Exp Biol Med 178:339–61

Forage RG, Ring JM, Brown RW, McInerney BV, Cobon GS, Gregson RP, Robertson DM, Morgan FJ, Hearn MTW, Findlay JK, Wettenhall REH, Burger HG, deKretser DM, 1986. Cloning and sequence analysis of cDNA species coding for the two subunits of inhibin from bovine follicular fluid. Proc Natl Acad Sci USA 83:3091–95

Gordon WL, Liu WK, Ward DN, 1986. Inhibin fractionation: a comparison of human and porcine follicular fluid, with particular reference to protease activation. Biol Reprod 35:209–18

Hartley BS, 1976. Strategy and tactics in protein chemistry. Biochem J 119:805–22

Huang ES-R, Miller WL, 1984. Porcine ovarian inhibin preparations sensitize cultured ovine gonadotrophs to luteinizing hormone-releasing hormone. Endocrinology 115:513–19

Johansson J, Sheth A, Cederlund E, Jornvall H, 1984. Analysis of an inhibin preparation reveals apparent identity between a peptide with inhibin-like activity and a sperm-coating antigen. FEBS Lett 176:21–26

Kemp BE, Niall HD, 1984. Relaxin. Vitam Horm 41:79–115

Kohan S, Froysa B, Cederlund E, Fairwell T, Lerner R, Johansson J, Kahn S, Ritzen M, Jornvall H, Cekan S, Diczfalusy E, 1986. Peptides of postulated inhibin activity. FEBS Lett 199:242–48

Li CH, Hammonds G Jr, Ramasharma K, Chung D, 1985. Human seminal α inhibins: isolation, characterization, and structure. Proc Natl Acad Sci USA 82:4041–44

Lilja H, Jeppsson J-O, 1985. Amino acid sequence of the predominant basic protein in human seminal plasma. FEBS Lett 182:181–84

Ling N, Ying S-Y, Ueno N, Esch F, Denoroy L, Guillemin R, 1985. Isolation and partial characterization of a M_r 32,000 protein with inhibin activity from porcine follicular fluid. Proc Nad Acad Sci USA 82:7217–21

Mason AJ, Hayflick JS, Ling N, Esch F, Ueno N, Ying S-Y, Guillemin R, Niall H, Seeburg PH, 1985. Complementary DNA sequences of ovarian follicular fluid inhibin show precursor structure and homology with transforming growth factor-β. Nature 318:659–63

McCullaugh DR, 1932. Dual endocrine activity of the testis. Science 76:19–20

Miyamoto K, Hasegawa Y, Fukuda M, Nomura M, Igarashi M, Kangawa K, Matsuo H, 1985. Isolation of porcine follicular fluid inhibin of 32K daltons. Biochem Biophys Res Commun 129:396–403

Ramasharma K, Sairam MR, Seidah NG, Chretien M, Yamashiro D, Li CH, Manjunath P, Schiller PW, 1984. Isolation, structure, and synthesis of a human seminal plasma peptide with inhibin-like activity. Science 223:1199–202

Rivier J, McClintock R, Spiess J, Vaughan J, Dalton D, Corrigan A, Azad R, Vale W, 1984. Isolation from porcine follicular fluid of a protein exhibiting potent inhibin-like biological activity. Proc 7th Intl Cong Endocrinol 655:1141–44

Rivier J, Spiess J, McClintock R, Vaughan J, Vale W, 1985. Purification and partial characterization of inhibin from porcine follicular fluid. Biochem Biophys Res Commun 133:120–27

Robertson DM, deVoss FL, Foulds LM, McLachlan RI, Burger HG, Morgan FJ, Hearn MTW, deKretser DM, 1986. Isolation of a 31 kDa form of inhibin from bovine follicular fluid. Mol Cell Endocr 44:271–77

Robertson DM, Foulds LM, Leversha L, Morgan FJ, Hearn MTW, Burger HG, Wettenhall REH, deKretser DM, 1985. Isolation of inhibin from bovine follicular fluid. Biochem Biophys Res Commun 126:220–26

Ryan BF, Joiner BL, Ryan TA Jr, 1985. Minitab, Boston, MA: Duxbury Press, pp. 193–217

Seidah NG, Arbatti NJ, Rochemont J, Sheth AR, Chretien M, 1984. Complete amino acid sequence of human seminal plasma β-inhibin. FEBS Lett 175:349–55

Sheth AR, Arabatti N, Calquist M, Jornvall H, 1984. Characterization of a polypeptide from human seminal plasma with inhibin (inhibition of FSH secretion)-like activity. FEBS Lett 165:11–15

Yamashiro D, Li CH, Ramasharma K, Sairam MR, 1984. Synthesis and biological activity of human inhibin-like peptide-(1-31). Proc Natl Acad Sci USA 81:5399–402